REMARKS

Reconsideration is respectfully requested in view of the foregoing remarks which follow.

The claims presently pending in the application are 1-31, 34-45, 55-75 and 79-86.

Claims 1-31, 34-45, 55-75, and 79-86 stand rejected under 35 U.S.C. § 103(a) as being unpatentable over Luo et al. (Bioconjugate Chem. 1999, 10, 755-763), in view of Sparer et al. (Controlled release delivery systems, Chapter 6, 1983, 107-119), Li et al. (US 5,977,163), and Desai et al. (US 5,648,506). This rejection is respectfully traversed.

Applicants disagree and take issue with the Examiner's reasoning in his § 103(a) rejection and hereby submit further arguments in support to the non-obviousness of the rejected claims.

The Examiner rejected all of the previously presented Claims as being obvious over Luo et al. in view of the fact that the latter discloses a HA-Taxol conjugate wherein HA and Taxol are covalently bonded by means of adipic dihydrazide (ADH). The results reported the conjugate disclosed by the Luo et al. are deemed by the Examiner to be significant and promising enough to warrant further investigation by one of ordinary skill in the art.

Applicants strongly disagree with this position and in support of the non-obviousness of the claimed invention ask that the following arguments be carefully considered.

With reference to Figure 8 of Luo et al., Ms Zanellato and Ms Campisi stated, in the Declaration submitted on April 18, 2008, that:

"*From this diagram reporting* in *ordinates the cell viability (%) and in abscissae the concentration of Taxol equivalent (μg/ml), it results that the* IC_{50} *of the HA-Taxol*

containing a Taxol loading of 5% is 0.05 μg/ml versus a IC_{50} for Taxol as such of about 0,11 μg/ml, thus indicating that this conjugate is at most twice more effective than to have Taxol as such."

The Examiner is believed to have arrived at his conclusions on the basis of a misunderstanding of the quoted extract.

In fact, looking at Table 3 of Luo et al. (page 761), preparation 3, which the quoted sentence from the Declaration above refers to, shows that in the case of HCT-116 colon cancer cells, the conjugate (5% Taxol loading) has IC_{50} of 0.52 μg/ml, corresponding to 0.052 μg/ml of free Taxol. The same values are indicated in Figure 8, wherein the authors compared the IC_{50} in terms of "concentration of Taxol equivalents (μg/ml)". From this Figure, the HA-Taxol conjugate with 5% Taxol loading showed an IC_{50} value of 0.052 μg/ml as concentration of Taxol equivalent, whereas the free Taxol has an IC_{50} value of 0.11 μg/ml as concentration of Taxol equivalent.

Following the cytotoxicity assessment method used in Luo et al. i.e., the IC_{50} based on Taxol equivalents, Applicants provided in the above-mentioned Declaration evidence proving the unexpectedly improved values given by the conjugates according to the current invention.

The reasoning reported in the Declaration has been that, **when the Taxol equivalents are used as a reference parameter**, the prior art teaches:

	IC_{50} (μg/ml)
Free Taxol	0.11
Conjugate of Luo et al.	0.052

This means that the IC_{50} concentration of 0.52 μg/ml of the prior art conjugate corresponds to 0.052 μg/ml as Taxol equivalent compared with 0.11 μg/ml of free Taxol as Taxol equivalent. When viewed in this perspective, the Declaration states that the conjugate of Luo et al. is *at most more than twice as effective as* the free Taxol.

However, in the same Declaration, the conjugates of the current invention have been proven to be more highly effective than the free Taxol.

Cells lines	Free Taxol	HYTAD2p20	HYTAD1p20	HYTAD2p10
MCF/7	3.5 nM	**0.11 nM**	**0.0022 nM**	**0.023 nM**
MDA/MB/231	0.35 μM	**0.34 nM**		**8.16 nM**
MDA/MB/468	9.4 nM		**0.016 nM**	
SKBR/3	0.23 nM			**0.0047 nM**

It follows, therefore, that the conjugates of the claimed invention are, respectively, more effective than free Taxol, the cytotoxicity being expressed in terms of Taxol equivalents:

- MCF/7 cells respectively:
  - 3.5/0.11 = **31.8 times**;
  - 3.5/0.0022= **1590 times**;
  - 3.5/0.023 = **152.1 times**,
- MDA/MB/231 respectively:
  - 350/0.34 = **1100 times**;
  - 350/8.16 = **43 times**;
- MDA/MB/468 respectively:
  - 9.4/0.016 = **587.5 times**;
- SKBR/3:
  - 0.23/0.0047 = **48.94 times**.

Notwithstanding this **evident, huge and unexpected improvement** with respect to the prior art conjugate, the Examiner has still rejected the claims on the basis that the results of the prior art, even if they are poorly significant, nonetheless, call for a further investigation by the skilled person. In the Examiner's opinion, "*the result is not promising only if the said HA-Taxol conjugate is less active compared to Taxol itself.*"

Applicants, in order to definitely and clearly establish to the Examiner's satisfaction the non-obviousness of the claimed invention, provide herein a **direct comparison of the IC_{50} values** of the free Taxol, the prior art conjugate and the conjugates of the invention.

Starting from the Luo et al. reference, the skilled person has knowledge of the following:

	IC_{50} (μg/ml)
Free Taxol	0.11
Conjugate of prior art	0.52

This means that in order to have the same result as the free Taxol, the conjugate of the prior art must be present in a concentration which is five (5) times greater. Therefore, the conjugate of the prior art as such is **fivefold less effective** than the free Taxol as such, when directly compared to each other (ratio free Taxol/conjugate = **0.21 times**).

This **result** is **unambiguously highly negative,** not only in terms of lack of effectiveness, but also in terms of the amount to be administered to a patient, that in this case must be increased fivefold with respect to Taxol.

Therefore, **a skilled person** confronting this teaching of Luo et al. **would never have considered the same "promising"** nor would he or she have found any motivation to undertake research which would lead in the direction of the claimed invention.

On the contrary, notwithstanding this **clear teaching away**, the **conjugates according to pending Claim 1** have been surprisingly proven to be **extremely more effective** than the free Taxol, as shown in Example 2 of the specification:

Cells lines	**Free Taxol**	**HYTAD2p20**	**HYTAD1p20**	**HYTAD2p10**
MCF/7	3.5 nM	**0.86 nM**	**0.024 nM**	**0.68 nM**
MDA/MB/231	0.35 μM	**2.58 nM**		**0.24 μM**
MDA/MB/468	9.4 nM		**0.18 nM**	
SKBR/3	0.23 nM			**0.14 nM**

It follows, therefore, that the conjugates of the invention are, respectively, more effective than free Taxol on:

- MCF/7 cells respectively:
  - 3.5/0.86 = 4.07 times;
  - 3.5/0.024= **145.83 times**;
  - 3.5/0.68 = 5.15 times,

- MDA/MB/231 respectively:
  - 0.35/0.00258 = **135.66 times**;
  - 0.35/0.24 = 1.46 times

- MDA/MB/468 respectively:
  - 9.4/0.18 = **52.22 times**;

- SKBR/3:
  - 0.23/0.14 = 1.64 times

As is immediately evident, **not only was there no expectation of success in view of the prior art teaching away from the claimed invention, but also the results achieved by the claimed invention have demonstrated that the claimed conjugates have cytotoxicity levels which are always higher than free Taxol, in fact, almost 150 times better than free Taxol**.

Additionally, the conjugates of the claimed invention are **advantageously water-soluble** notwithstanding their high molecular weights, whereas the prior art stated that the solubility decreases when increasing the molecular weight.

As a matter of fact, Luo et al. at page 760, left column, second paragraph, states that low molecular weight hyaluronic acid (LMW HA), particularly having a MW of 11,199 Da, has been selected for many reasons, but mainly because only low molecular weights HA can be cleared by the kidney and only LMW materials would suffer minimal further degradation in plasma and would be rapidly taken up by cells.

Furthermore, the skilled person, by taking into account Table 2, Table 3 and page 759, last seven lines of Luo et al. would have been informed that:

- by increasing the Taxol loading, the resulting conjugate shows decreased solubility in water, thus leading to a reduced cytotoxicity (see page 762 lines 3-7); and,

- the tests were carried out in a solvent mixture of DMSO/H_2O.

The skilled person is therefore made expressly aware of the fact that an increased molecular weight and/or Taxol loading involve problems of decreased solubility and effectiveness.

Unexpectedly and advantageously, the conjugates of the current invention, not only show **very high effectiveness**, as above demonstrated, but also **very good solubility** even when a HA of 200,000 Da is selected and even if a high Taxol loading is present, as disclosed in Examples 2, 7, 10 and 12.

Particularly, Example 10 relates to the preparation of an ester derivative of hyaluronic acid with esterification at the carboxyl of about 30% w/w, and the last sentence thereof recites: "... the filamentous product thus obtained is dissolved in water and dialysed and lastly freeze-dried."

Moreover, Example 12 concerns tests of solubility of the conjugate obtained according to Example 7, i.e. an ester derivative of Ha with paclitaxel with esterification at the carboxyl of 16.3% w/w, starting from HA with a molecular weight of 200,000 Da. In particular, it refers to a 5% aqueous solution of glucose wherein said HA-paclitaxel conjugate is present in a surprisingly high concentration of 32.8 mg/ml.

In view of the above arguments, the skilled person from a reading of Luo et al. would only know that the sole conjugate disclosed therein is definitely undesirable in many respects, namely:

- the **conjugate** of Luo et al. **as such** is **fivefold less effective** than the free Taxol as such, when directly compared to each other (ratio free Taxol/conjugate = **0.21 times**);

- the HA must have low molecular weights, otherwise the effectiveness disadvantageously decreases; and,

- the cytotoxicity decreases as the Taxol loading increases, since "***high loading decreases the solubility of HA-Taxol conjugate and thus limits the cytotoxicity of the conjugate relative to that of the free drug***" (Luo et al. page 762, lines 1-7).

Therefore, the skilled person would necessarily be led to believe that a result which is **fivefold less effective** than the free Taxol is the best possible result for this kind of conjugate, since **any change that may be supposed is already addressed as exacerbating the situation**, such as by increasing the MW of HA the effectiveness decreases and by increasing the Taxol loading the solubility decreases, all these aspects **negatively affecting the cytotoxicity**.

Therefore, the person of ordinary skill in the art would **never have considered** the information disclosed in **Luo et al. as promising.** It is respectfully submitted that for a scientist of ordinary skill in the art to consider Luo et al.'s data as promising, would be ***contrary to the scientific process.***

Sparer et al. disclose glycosaminoglycans drug complexes, i.e. cysteine-GAG and chloramphenicol-GAG complexes. It is well known that GAGs are a family of sulfated sugar chains of long unbranched polysaccharides consisting of a repeating disaccharide unit. Subtle variations in stereochemistry, length, and patterns of sulfation differ between and within GAG families.

Sparer et al. not only does not address HA as such, but also, as is well recognized even by the Examiner, never discloses Taxol and the specific technical aspects related thereto, for instance, its loading in general and to its loading on HA, especially in view of the cytotoxic activity thereof. Therefore, the **skilled person would have never deemed Sparer et al. to be relevant prior art,** and even more so, **would never have had any motivation to overturn what is clearly taught by Luo et al. with reference to HA-Taxol conjugate cytotoxicity and effectiveness**.

Li et al. refers to paclitaxel and docetaxel complexes with polyethylene glycol polymers. The Examiner is of the opinion that "*even though Li et al. do not teach Taxol hyaluronic acid conjugates, one of ordinary skill in the art would recognize from their teaching that conjugates containing HA and Taxol can also be used in a method for*

treating of cancers, tumours and restenosis and for coating medical devices" (page 7, lines 5-8, of the outstanding Office Action). However, **the skilled person would have never recognized that conjugates containing HA and Taxol can also be used, since Luo et al. discourages them from being considered desirable**, as amply demonstrated from the foregoing.

Desai et al. refers to Taxol complexes with polyethylene glycols, similar to Li et al. Analogously, the Examiner states that, even if this document does not exemplify Taxol-HA conjugates, the skilled person could have used the process disclosed in Desai et al. for producing the same. However, as explained above, for Li et al., **the skilled person would have never recognized the desirability of conjugates containing HA and Taxol, since Luo et al. discourage such a consideration**.

It is respectfully submitted that the facts established by Applicants' Rule 132 Declaration, which unequivocally demonstrates unexpected results, successfully rebuts the Examiner's determination of obviousness based on a "preponderance of the evidence standard", which simply requires that the evidence submitted [by Applicants] be more convincing than the evidence offered in opposition to it. *In re Oetiker*, 24 USPQ2d 1443 (Fed. Cir. 1992).

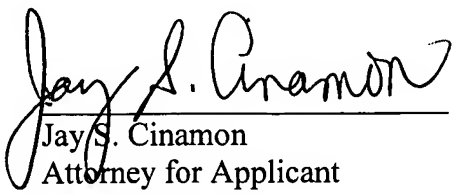
For all the above reasons, Applicants are convinced that the claimed conjugates are new and unobvious and distinguish over the combined teachings of the art. Withdrawal of the § 103(a) rejection is solicited since a *prima facie* case of obviousness has clearly not been established.

The timely issuance of a Notice of Allowance is respectfully requested.

Please charge any fees which may be due and which have not been submitted herewith to our Deposit Account No. 01-0035.

Respectfully submitted,

ABELMAN, FRAYNE & SCHWAB
Attorneys for Applicant

By 
Jay S. Cinamon
Attorney for Applicant
Reg. No. 24,156

666 Third Avenue
New York, NY 10017-5621
Tel.: (212) 949-9022
Fax: (212) 949-9190